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Study of the Stability of Creatine Kinase in Control Materials

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Summary: We examined the stability of twelve control materials from different suppliers containing creatine kinase. The stability was assessed storing preparations at 4 °C, 27 °C and 37 °C and periodically measuring creatine kinase catalytic concentration. The activity was significantly greater when the lyophilized material was reconstituted with water at 4 °C as compared to 27 °C and 37 °C. All preparations tested showed good stability at 4 °C over a 72 hour period. We considered the suitability of tested control materials for quality-control purposes.

Introduction

The introduction of control materials into the clinical laboratory helped improve the precision of measurements and permitted the performance of the recommended methods to be assessed. However, there are some problems associated with control preparations that should be considered. The most important is the instability of the enzymatic components, especially creatine kinase and alkaline phosphatase. Several studies on stability of creatine kinase[1]) in control materials have been reported in the literature (1–9). Apparently, the stability of creatine kinase in solution can be affected by matrix composition (albumin, ADP, EDTA ...), enzyme source (human, porcine, rabbit ...), isoenzyme composition (MB, MM or BB), temperature, light, storage conditions and sulphydryl compounds among others.

In order to test different control materials for use in the quality-control of creatine kinase analysis, we carried out a stability study of 12 currently used lyophilized and liquid preparations which contain creatine kinase. The stability was assessed by storing the preparations at 4 °C, 27 °C and 37 °C and periodically measuring the enzymatic activity. The effect of the temperature of the diluent in the reconstitution step of lyophilized materials was also examined.

Materials and Methods

Specimens

Eleven commercial control sera containing creatine kinase and control material from a national quality-control programme were used.

The materials and suppliers were:

Accutrol A from Sigma (St. Louis, MO, USA);
Control Serum P from Roche (Basel, Switzerland);
Decision level II from Beckman (Fullerton CA, USA);
Lyphocheck level 2 from Bio-Rad (Anaheim, CA, USA);
Multisera E from Randox (Crumlin, N. Ireland);
Par level 3 from Medical Analysis Systems (Camarillo, CA, USA);
Plus 33 from Biotrol (Paris, France);
Precipath U from Boehringer Mannheim (Mannheim, Germany);
Qualitrol H from Merck (Darmstadt, Germany);
Serodos P from Human (Taunusstein, Germany);
Validate A from Organon Teknika (Eppelheim, Germany) and
Control sera level 4 from SEQC (Sociedad Española de Bioquímica Clínica y Patología Molecular, Spain).

Decision II and Par 3 were liquid materials, whereas the rest was lyophilized.

Creatine kinase determinations

Creatine kinase activity was determined using a commercial kit from Boehringer Mannheim (Mannheim, Germany) at 37 °C

[1]) Enzymes:
Creatine kinase, ATP : creatine N-phosphotransferase, EC 2.7.3.2

following the manufacturer's instructions (according to ECCLS) in a spectrometer Uvikon 860 (Kontron Instruments, Zürich, Switzerland).

Stability study

Lyophilized control materials were reconstituted with distilled water at 37 °C, 27 °C and 4 °C and following the manufacturer specifications. Vials were then stored at the respective temperatures for at least 72 hours. Creatine kinase catalytic concentration was measured after 30 min of reconstitution (time 0), and after 1, 2, 3, 24, 48 and 72 hours after reconstitution. Activity was measured in duplicate in each vial.

Results and Discussion

Figures 1 through 3 show the results obtained in the stability study. The materials were stable for at least 24 hours at 4 °C and only one of them showed a significant decrease of creatine kinase activity (12%) after 72 hours.

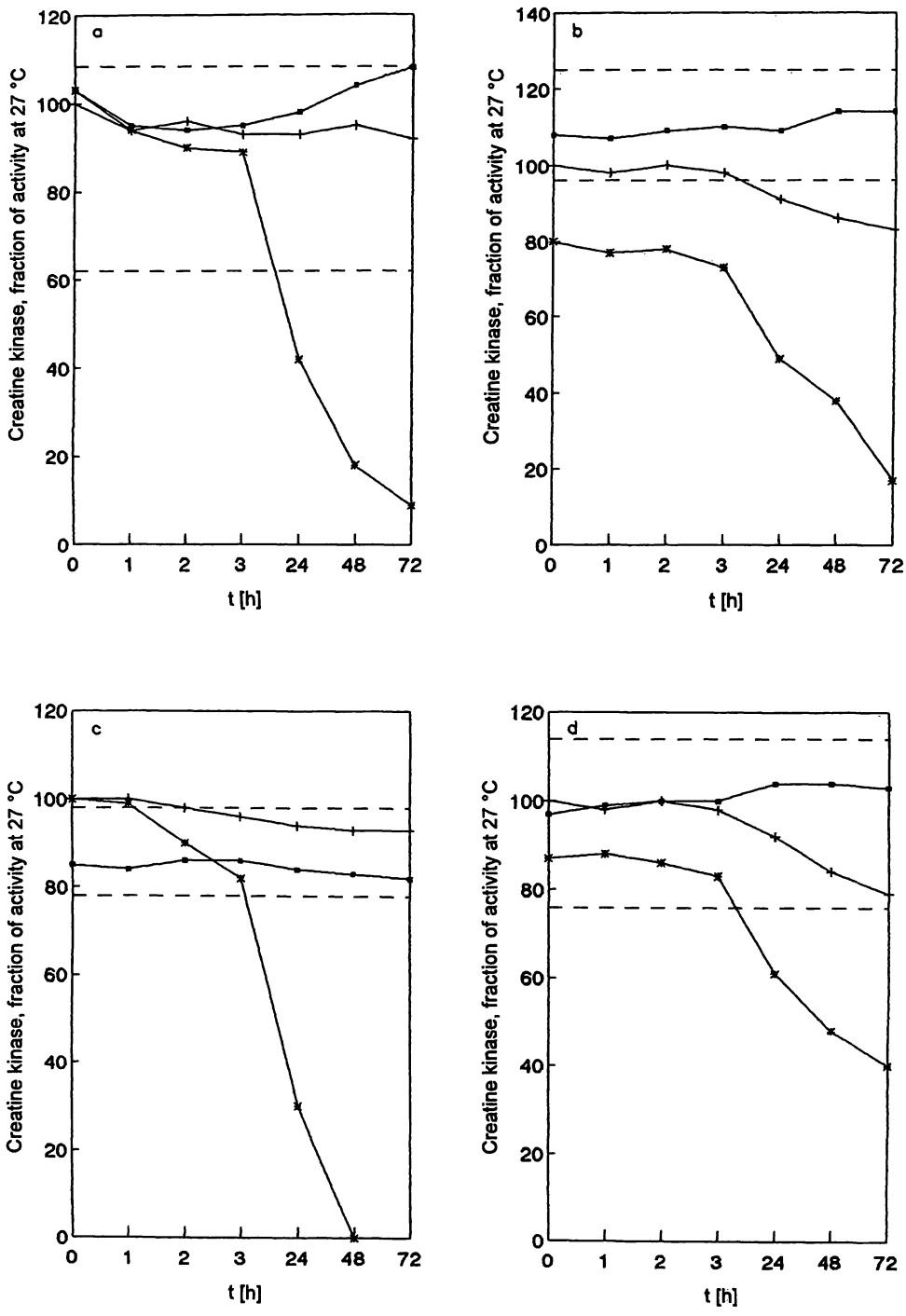

Fig. 1 Stability study of creatine kinase activity in
a) Accutrol A (Sigma),
b) Control Serum P (Roche),
c) Decision II (Beckman) and
d) Lyphocheck 2 (Bio-Rad).

Vials were reconstituted and stored at 4 °C (■), 27 °C (+) and 37 °C (*).
Creatine kinase activity at each time and temperature is in reference to the activity obtained at 27 °C after reconstitution. Values are the mean of duplicates. Broken lines indicate the range of values stated by the supplier.

All materials except two (Multisera E and SEQC 4) were stable for at least 3 hours at 27 °C. Creatine kinase activity in the materials stored at 37 °C decreased at a higher rate in all but one (Par 3) which showed remarkably stability at elevated temperatures. Decreases in activity in six of the twelve controls ranged from 7 to 20% after 3 h at 37 °C. For two materials the decrease was higher than 50% and for four lower than 5% under the same conditions. The stabilized liquid control materials were generally more stable than the freeze-dried materials once reconstituted, with the exception of Decision II stored at 37 °C.

The creatine kinase activity measured in most of the studied materials was influenced by the temperature of the water used to reconstitute them. This effect was first described by *Feld* et al. (1) using control sera from three different suppliers. Here we show that this phenomenon

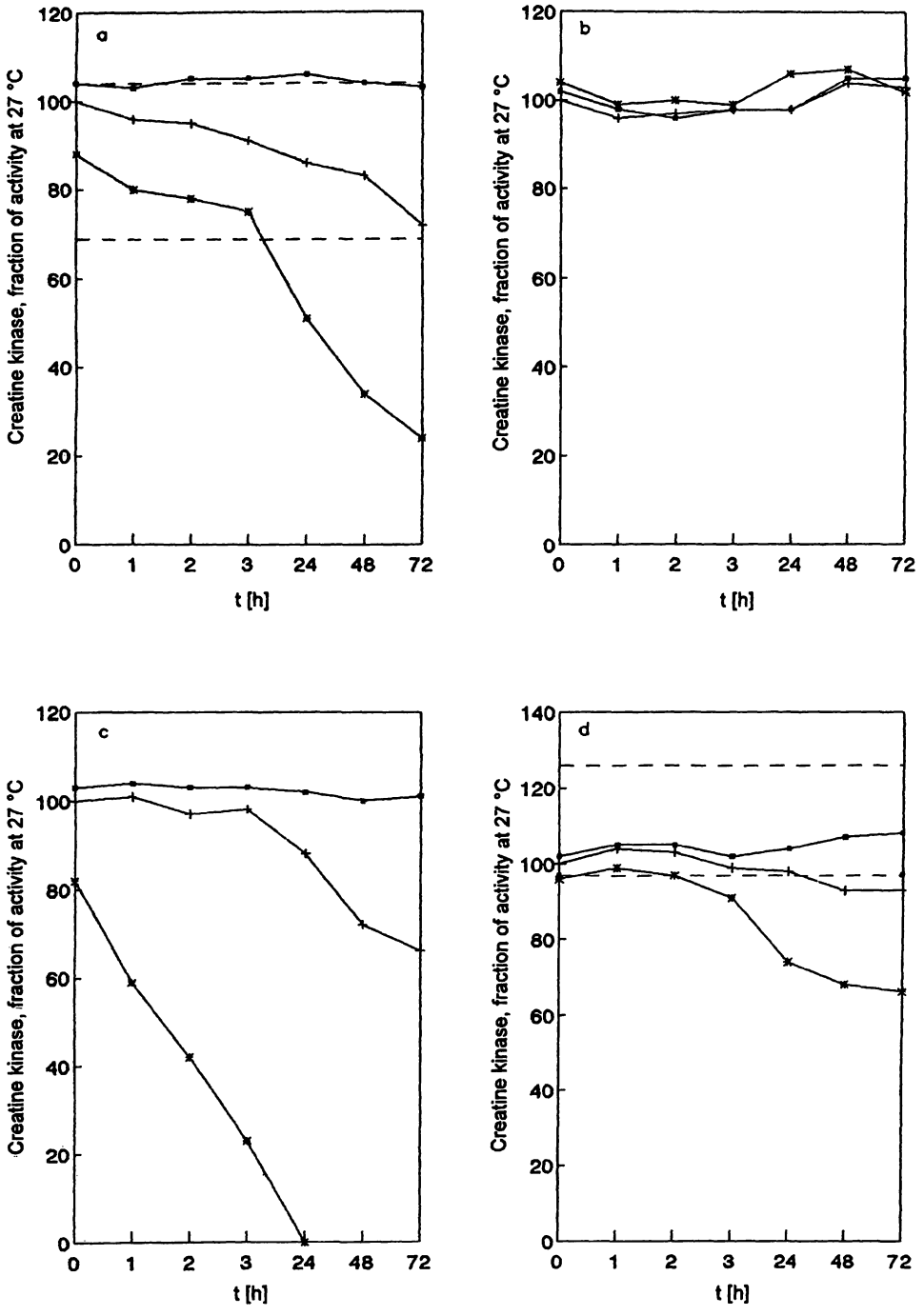

Fig. 2 Stability study of creatine kinase activity in
a) Multisera E (Randox),
b) Par 3 (Medical Analysis Systems),
c) Plus 33 (Biotrol) and
d) Precipath U (Boehringer Mannheim).

Vials were reconstituted and stored at 4 °C (□), 27 °C (+) and 37 °C (*).
Creatine kinase activity at each time and temperature is in reference to the activity obtained at 27 °C after reconstitution. Values are the means of duplicates. Broken lines indicate the range of values stated by the supplier.

is also observed in many other materials currently used in Europe. Creatine kinase activity measured in materials reconstituted at 4 °C may be up to 35% higher than that measured in the same materials reconstituted at 37 °C (Qualitrol H, Validate A, SEQC 4). Creatine kinase contained in Accutrol A, Precipath U and Serodos P was not sensitive to the temperature of the water used to reconstitute the material. Very often control materials are reconstituted using distilled water at room temperature which may vary considerably depending on the calibration temperature of the glassware, on the geographic location and on the season of the year. Internal and external quality control expect lyophilized materials to have constant creatine kinase activity. The variation of activity caused by the temperature of the water used to reconstitute the material will increase the variance observed in the control of creatine kinase measurements.

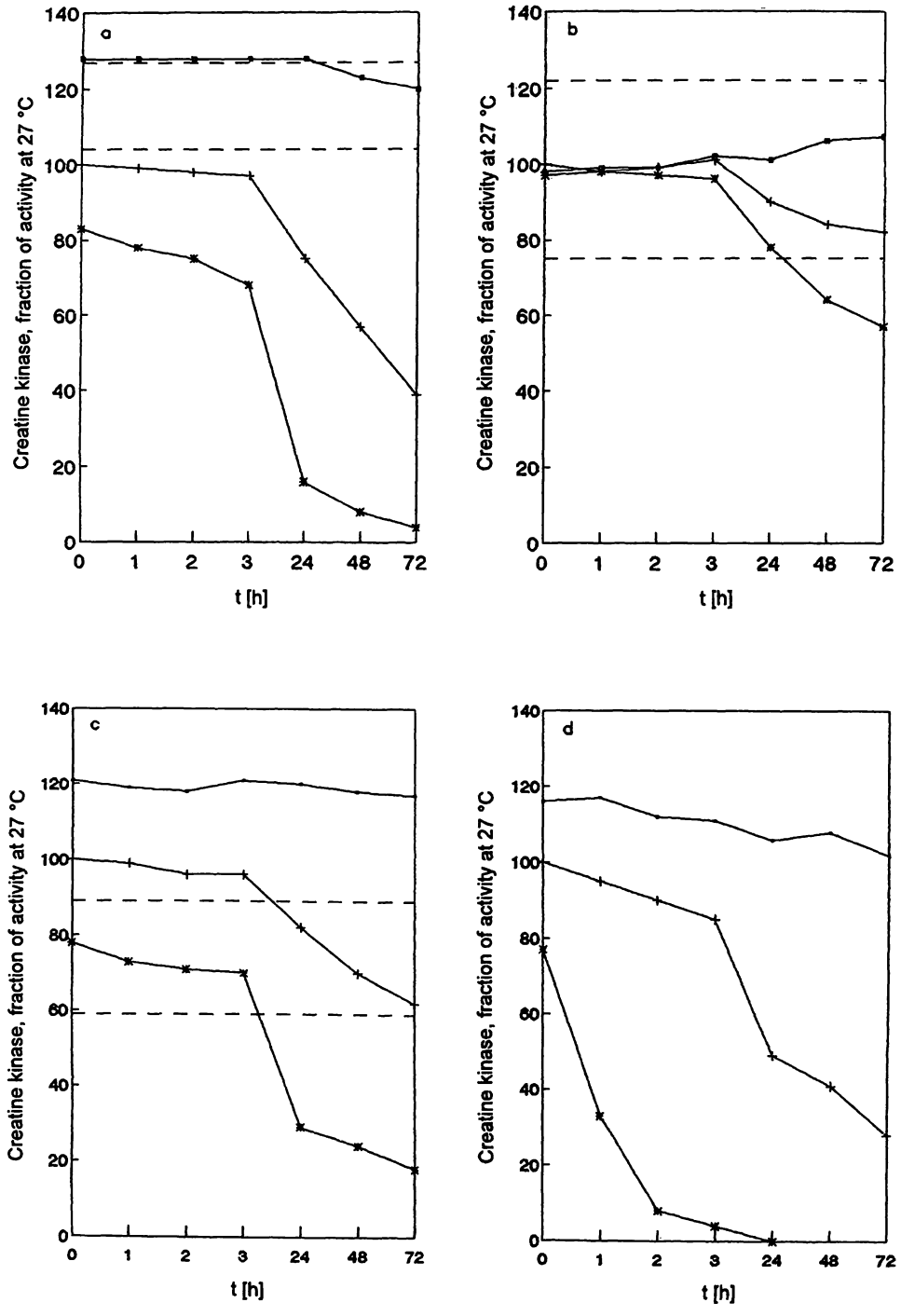

Fig. 3 Stability study of creatine kinase activity in
a) Qualitrol H (Merck),
b) Serodos P (Human),
c) Validate A (Organon Teknika) and
d) SEQC 4.

Vials were reconstituted and stored at 4 °C (■), 27 °C (+) and 37 °C (*).
Creatine kinase activity at each time and temperature is in reference to the activity obtained at 27 °C after reconstitution. Values are the mean of duplicates. Broken lines indicate the range of values stated by the supplier.

The range of values for creatine kinase activity assigned by the manufacturer comprised the obtained activity for materials reconstituted at 4 °C or at 27 °C. Qualitrol H and Validate A were exceptions possibly due to the very important effect of the water temperature on reconstitution over the final creatine kinase activity obtained.

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